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14. ABSTRACT

During this grant period, we obtained evidence that eukaryotic elongation factor-2 kinase (eEF-2K) plays a key role in the regulation of cellular energy metabolism in breast cancer cells. We demonstrated that silencing of eEF-2K led to a greater reduction of cellular ATP and lactate. We further showed that suppression of eEF-2K impeded tumor cell growth in the serum/nutrient-deprived cultures, handicapped cell survival, and enhanced the efficacy of growth factor antagonist gefitinib and lapatinib, ER stressors thapsigargin and tunicamycin, in breast cancer cells. The results of the first grant year study have identified eEF-2K as a novel regulator of cancer cell metabolism, and provide new evidence that targeting eEF-2K may represent a novel approach to enhancing the effectiveness of anti-cancer reagents such as growth factor inhibitors and ER stress inducers.

15. SUBJECT TERMS
Elongation factor-2 kinase; breast cancer; energy metabolism; apoptosis

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Table of Contents

Page

INTRODUCTION

Breast cancer is one of the major causes of cancer mortality in women. Current therapies for breast cancer mainly target molecular signaling pathways that promote tumor cell proliferation and survival, facilitate angiogenesis or block cellular differentiation. An unexplored approach to breast cancer treatment is targeting of cellular energetics, which has long been known to be aberrant in malignant cells; yet its importance in cancer biology and treatment has often been neglected. It was first noted by Dr. Otto Warburg that cancer cells rely mainly on aerobic glycolysis to generate ATP instead of more efficient mitochondrial oxidative phosphorylation, resulting in the increased rate of glucose uptake and lactate production even in the presence of sufficient oxygen supply (1). Eukaryotic elongation factor-2 kinase (eEF-2K), a negative regulator of protein synthesis (2), plays an important role in cell survival (3), and is overexpressed in breast cancer cell lines and human breast cancer specimens (4). In this study, we tested whether eEF-2K was involved in the regulation of energy metabolism in breast cancer cells, and whether targeting eEF-2K rendered breast cancer cells less capable of coping with metabolic/ therapeutic stress and render them more sensitive to treatments.

BODY

Training

Mentoring:

According to my training plan, I meet with my primary mentor weekly, and the co-mentor every two months to discuss project goals. In the past one year, I have also had a meeting with my mentoring committee once to discuss the research plan and progress.

Management and Career Independence:

In the past grant period, I attended the following courses:

Penn State Hershey Post-doctoral Society Workshop: Career Transition Planning **(**December 10, 2011)

Scientific Management Series (September 25, 2012)

Future Faculty Seminar (April 20, 2012)

Courses and Training Programs:

In the past grant period, I attended the following courses and program:

Biochemistry 510, Penn State College of Medicine

AACR Annual Meeting (March 31~April 4, 2012)

Annual Penn State Hershey Cancer Institute Symposium (May 10, 2012)

Annual Penn State College of Medicine Review Course for Clinical Oncologists (July 20, 2012)

Translational Research Symposium of Penn State Hershey Cancer Institute (June 8, 2012)

Cancer Institute Interdisciplinary Conference Series (monthly)

Department of Pharmacology Seminar Series (weekly)

The Experimental Therapeutics Program's Monthly Meeting

Penn State Cancer Institute Breast Cancer Focus Group Meeting and Penn State Cancer Institute & Division of Medical Oncology (weekly).

Researching

Task 1 To determine the importance of eEF-2K in energy metabolism of breast cancer cells.

Task 2 To determine the impact of targeting cellular energetics on breast cancer treatment.

To analyze whether eEF-2K plays a regulatory role in cellular energy metabolism in breast cancer cells, we silenced eEF-2K expression using siRNA in three human breast cancer cell lines (Fig. 1A), and then measured ATP and lactate

levels. As shown in Fig. 1B, inhibition of eEF-2 kinase by siRNA significantly decreased ATP level. As tumor cells rely mainly on glycolysis to generate energy instead of oxygen phosphorylation, and the level of lactate, and the final product of glycolysis, can be used to assay glycolytic activity (5), we compared the lactate levels in breast cancer cells with or without silencing of eEF-2K expression. We further found that inhibition of eEF-2K also resulted in reduction of lactate levels (Fig. 1C). These results further support a role for eEF-2K in activating glycolysis in breast cancer cells.

We next tested whether eEF-2K plays a prosurvival role in response of breast cancer cells to a compromised supply of nutrients and growth factors. We knocked down eEF-2 kinase, and then compared the growth and survival of the cells with or without silencing of eEF-2K in serum-free medium or HBSS. As shown in Fig. 2A, suppression of eEF-2K hindered the tumor cell growth in the absence of serum. Knockdown of eEF-2K also caused more death of MCF-7 cells cultured in HBSS (Fig. 2B).

To determine whether suppression of the eEF-2K alters sensitivity of tumor cells to anti-cancer treatment such as growth factor inhibitors that are in clinical use, we first transfected MDA-MB-468 cells with an eEF-2K-targeted siRNA or a non-targeting RNA, and then treated the transfected cells with a series of concentrations of gefitinib or lapatinib. Fig. 3 shows that silencing of eEF-2K expression increased sensitivity of MDA-MB-468 cells to gefitinib and lapatinib. Similar results were observed with the human breast cancer cells MCF-7. These results indicate that inhibition of eEF-2K sensitized breast cancer cells to growth factor inhibitors

As shown in Fig. 4A, depletion of EEF2K augmented apoptosis in breast cancer cells treated with ER stress inducers thapsigargin or tunicamycin, as determined by an increased Annexin V staining. Enhancement of ER stress-induced apoptosis by inhibition of eEF-2K was also evidenced by an increase in Bim level and a decrease in survivin level in the cells with silencing of eEF-2K expression, as compared with the cells transfected with a non-targeting siRNA (Fig. 4B).

KEY RESEARCH ACCOMPLISHMENTS

• We found that silencing of eEF-2K caused a reduction of cellular ATP and lactate in human breast cancer cells.

• We showed that inhibition of eEF-2K decreased growth and survival of metabolically stressed breast cancer cells.

• We observed that suppression of eEF-2K sensitized breast cancer cells to growth factor inhibitors.

• We found that inhibition of eEF-2K enhanced apoptotic activities breast cancer cells in response to ER stress.

REPORTABLE OUTCOMES

Abstract

Cheng Y, Zhang Y, Ren XC, Yang JM: The essential role of eEF-2K in determining fate of glioma cells under ER stress. **Proc Amer Assoc Cancer Res.** 2012. Abstract number: 4985.

Award

Penn State Hershey Annual Outstanding Postdoctoral Scholars and Fellows Award (Feb 21, 2012)

CONCLUSIONS

The results of the first year study provide new evidence that eEF-2 kinase is a positive regulator of cellular metabolism in breast cancer cells, and that targeting eEF-2K may represent a novel approach to enhancing the effectiveness of anti-cancer treatment such as growth factor inhibitors and ER stress inducers.

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APPENDIX

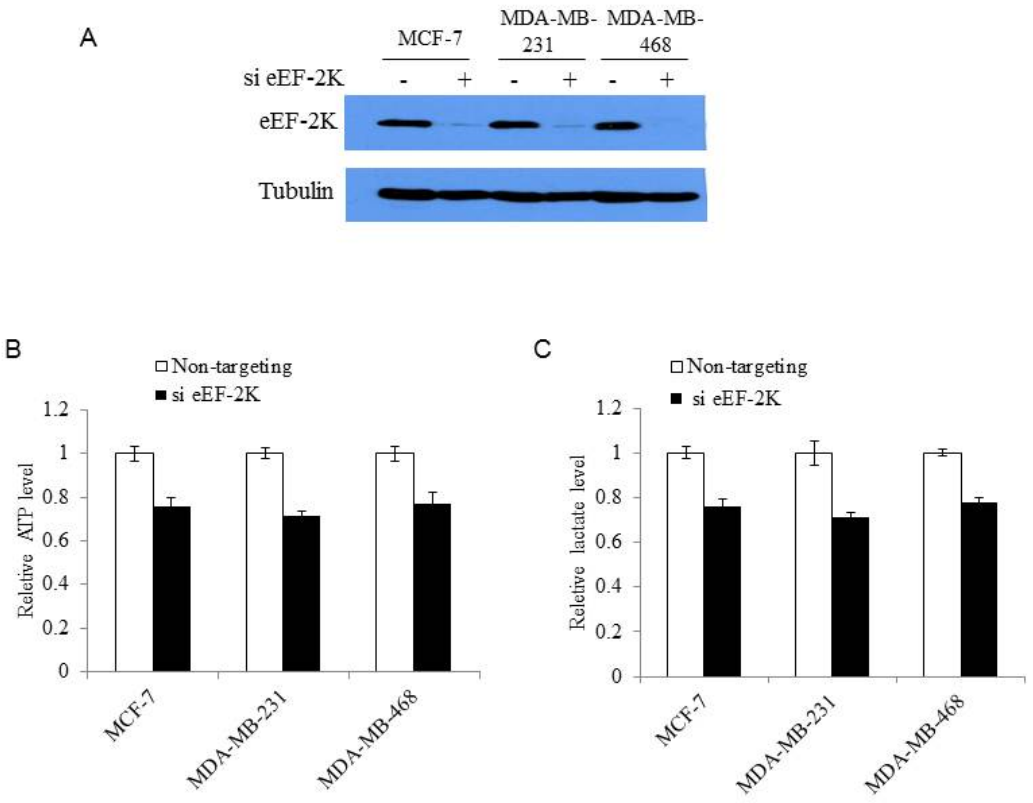

Figure 1. Effects of eEF-2K on the levels of ATP and lactate in human breast cancer cell lines. MCF-7, MDA-MB-231 and MDA-MB-468 cells were transfected with a non-targeting RNA or a siRNA targeting eEF-2 kinase. **(A)** The expression of eEF-2 kinase was determined by Western blot. Tubulin was used as a loading control. The ATP level **(B)** or the relative lactate level **(C)** was measured in cells with or without silencing of eEF-2K. Each bar represents the mean ± SE of three experiments.

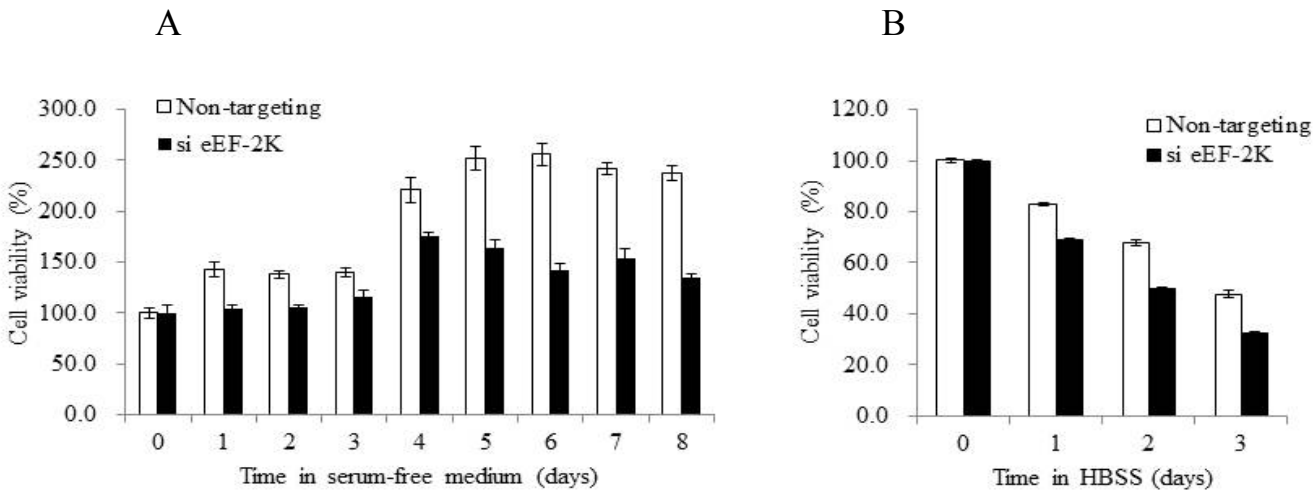

Figure 2. Effects of eEF-2K on survival of human breast cancer cells. MCF-7 cells were transfected with a non-targeting RNA or a siRNA targeting eEF-2 kinase, followed by serum-free medium incubation **(A)** or HBSS treatment **(B)** for the indicated time period, and cell viability was measured using MTT assay. Each bar represents the mean ± SE of three experiments.

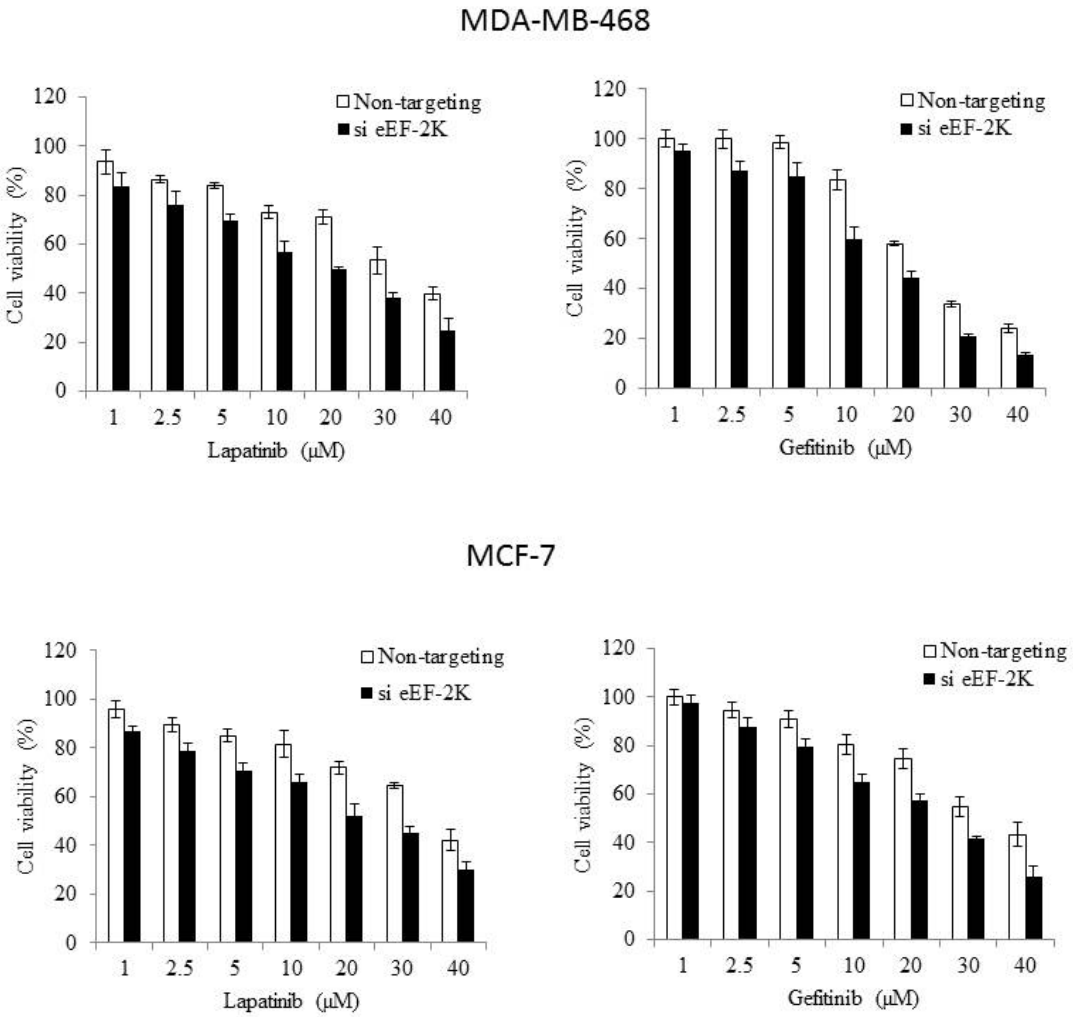

Figure 3. Effects of eEF-2 kinase silencing on sensitivity of human breast cancer cells to growth factor inhibitors. MDA-MB-468 and MCF-7 cells were transfected with an siRNA targeting eEF-2 kinase or a non-targeting RNA, and then treated with a series of concentrations of gefitinib or lapatinib for 48 h. At the end of treatment, cell viability was determined using MTT assay. Each bar represents the mean ± SE of three experiments.

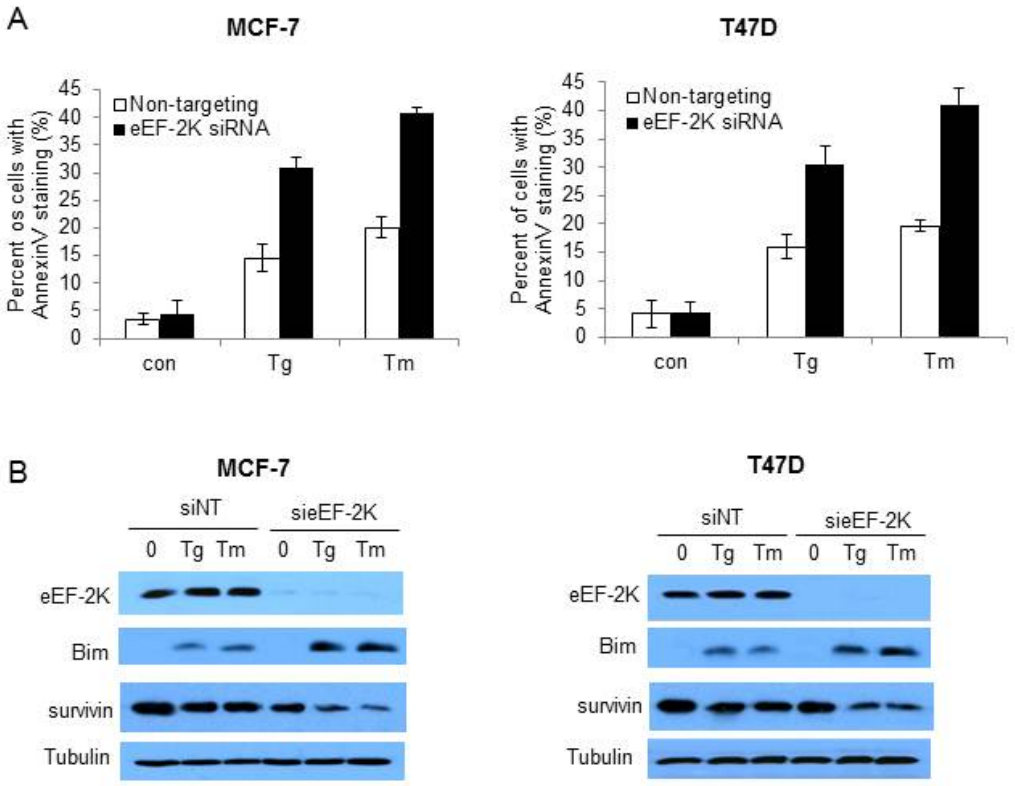

Figure 4. Silencing of eEF2K expression results in increased apoptosis in breast cancer cells in response to ER stress . MCF7 or T-47D cells were transfected with a non-targeting RNA or a siRNA targeting eEF2K, followed by treatment with thapsigargin (Tg) or tunicamycin (Tm) for 48h. **(A)** Apoptosis was determined by flow cytometric analyses of Annexin staining. Each bar represents the mean ± SE of three experiments. **(B)** The expression of eEF2K, Bim and survivin were examined by Western blot. Tubulin was used as a loading control.